

Video Article

# Extraction of Organochlorine Pesticides from Plastic Pellets and Plastic Type Analysis

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## Abstract

Plastic resin pellets, categorized as microplastics ( $\leq 5$  mm in diameter), are small granules that can be unintentionally released to the environment during manufacturing and transport. Because of their environmental persistence, they are widely distributed in the oceans and on beaches all over the world. They can act as a vector of potentially toxic organic compounds (e.g., polychlorinated biphenyls) and might consequently negatively affect marine organisms. Their possible impacts along the food chain are not yet well understood. In order to assess the hazards associated with the occurrence of plastic pellets in the marine environment, it is necessary to develop methodologies that allow for rapid determination of associated organic contaminant levels. The present protocol describes the different steps required for sampling resin pellets, analyzing adsorbed organochlorine pesticides (OCPs) and identifying the plastic type. The focus is on the extraction of OCPs from plastic pellets by means of a pressurized fluid extractor (PFE) and on the polymer chemical analysis applying Fourier Transform-Infrared (FT-IR) spectroscopy. The developed methodology focuses on 11 OCPs and related compounds, including dichlorodiphenyltrichloroethane (DDT) and its two main metabolites, lindane and two production isomers, as well as the two biologically active isomers of technical endosulfan. This protocol constitutes a simple and rapid alternative to existing methodology for evaluating the concentration of organic contaminants adsorbed on plastic pieces.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/55531/>

## Introduction

Global production of plastics is continuously rising since the 1950's to reach 311 million tons in 2014 with about 40% used in packaging<sup>1</sup>. In parallel, increasing quantities of these materials are accumulating in the environment, which might pose a serious threat to the ecosystems<sup>2</sup>. Although already reported in the 1970's, the occurrence of plastic debris in the marine environment has only received a greater attention in the past decade. Especially microplastics, plastic fragments with a diameter of  $\leq 5$  mm, are now recognized as one of the main marine water quality issues<sup>3</sup>.

Plastic resin pellets are small granules generally in the shape of a cylinder or a disk and with a diameter of a few mm (e.g., 2 to 5 mm)<sup>4,5</sup>. They fall in the category of microplastics. These plastic granules are industrial raw material from which final plastic products are manufactured through re-melting and molding at high temperature<sup>6</sup>. They can be unintentionally released to the environment during manufacturing and transport. For instance, they can be directly introduced to the ocean through accidental spills during shipping<sup>4,7,8</sup>. They can be carried from land to oceans by surface run-off, streams and rivers. Because of their environmental persistence, plastic pellets are widely distributed in the oceans and found on beaches all over the world<sup>4</sup>. They can negatively affect marine organisms and can enter the food chain, where their effects are unpredictable<sup>6,7</sup>. Furthermore, several studies have revealed the presence of environmental contaminants adsorbed onto plastic pellets collected in a coastal environment, which act as vector of these potentially toxic chemicals<sup>4,9,10</sup>. In fact, there is laboratory evidence suggesting that these chemicals can bioaccumulate in tissues of organisms after being released from ingested plastic fragments<sup>11,12</sup>.

In order to better assess the hazards associated with the occurrence of plastic pellets in the marine environment, it is necessary to develop methodologies that can determine sorbed organic contaminants. An important step is the extraction of the chemicals from the plastic matrices, which can present heterogeneous physical-chemical characteristics depending on the polymer type, its degradation stage, and pre-treatments. Most of the investigations reported in the literature use maceration or Soxhlet techniques<sup>4,5,6,9,13,14,15,16,17,18</sup>, which are solvent and/or time consuming. Regarding the growing interest for this issue, alternatives should be developed, for a faster evaluation of organic contaminants adsorbed on plastic pieces. In addition, plastic chemical analysis provides information about the chemical structure of the microplastics. As a result, the predominant types of polymers and copolymers present in the environment can be evaluated. Although plastic fragments are usually made of polyethylene (PE) and polypropylene (PP)<sup>5</sup>, some sampling locations can present a particular profile where other categories

are significantly represented (e.g., ethylene/vinyl acetate copolymer and polystyrene (PS)). FT-IR spectroscopy is a reliable and user-friendly technique for polymer identification commonly used to identify microplastics<sup>19,20</sup>.

The main aim of the present work is to offer a rapid and simple option for extracting OCPs and related compounds from plastic pellets by means of a PFE. However, the design of the protocol includes all steps leading to the determination of sorbed OCPs, from the sampling of the resin pellets to the analysis of the compounds. The method of identifying the plastic type is also described. The developed methodology focuses on 11 OCPs and related compounds: i) DDT (2,4'- and 4,4'-dichlorodiphenyltrichloroethane) and its two main metabolites DDE (2,4'- and 4,4'-dichlorodiphenyldichloroethylene) and DDD (2,4'- and 4,4'-dichlorodiphenyldichloroethane); ii) the isomer gamma-hexachlorocyclohexane ( $\gamma$ -HCH) as the main ingredient of the pesticide lindane and the two isomers  $\alpha$ -HCH and  $\beta$ -HCH released during its production<sup>15</sup>; iii) and the two biologically active isomers endosulfan I (Endo I) and II (Endo II) present in the technical endosulfan. The studied pesticides are broad-spectrum insecticides, chemically stable, hydrophobic, and classified as persistent organic pollutants (POPs) by the Stockholm Convention<sup>21</sup>.

## Protocol

### 1. Plastic Pellet Sampling

- Before going to the field, triple rinse all required sampling materials (e.g., tweezers and aluminum foil) with acetone or ethanol (99%). In case the material cannot be solvent-rinsed, heat it at 450 °C overnight in an oven (e.g., glassware).  
NOTE: In tourist areas, obtain information about possible beach cleaning activities that would remove most of the marine litter including microplastics. If possible, plan the sampling ahead of this operation. If sampling during the clean-up season, specify the details of this activity in the identity form (e.g., dates, clean-up method used, etc.)
- Wearing gloves, collect plastic pellets from the beach with solvent-rinsed stainless steel tweezers.
- Sample 50 to 100 pellets per location, which corresponds to 5 to 10 replicates per location (10 pellets per replicate). If the required number of pellets cannot be obtained, collect the maximum pellets possible and specify it in the identity beach form.
- At the end of the sampling, wrap the collected pellets in solvent-rinsed aluminum foil. Glass bottles can be used as an alternative or even paper bags.
- Fill in the identity form of the selected beach with the missing information (i.e., beach location, weather conditions, details on pellets, etc.).
- Transport the samples to the laboratory in an icebox if the ambient temperature exceeds 25 °C. This step can be skipped in the case of short trips (e.g., <1 h).
- Once in the laboratory, gently wipe off removable particles (e.g., sand) of the pellets. Dry the samples if necessary in a desiccator prior to storage (darkness, T <25 °C). Avoid rooms where OCPs might be in use (e.g., storage of standard solutions).
- Store the pellets in the fridge (4 °C) for short periods (i.e., few days) or in the freezer (-18 °C) for longer periods in solvent-rinsed aluminum foil.
- Avoid exposure of samples to artificial light or sunlight. Handle the samples as little as possible before analysis to decrease the risk of contamination.

### 2. Extraction of OCPs from Plastic Pellets

- To reduce the risk of contamination, work in a clean laboratory using carefully washed glassware as follows: 2 rinses with analytical-grade acetone, dichloromethane and n-hexane. Dry the glassware under nitrogen flow and protect from contact with ambient air (e.g., cover with cleaned aluminum foil). Apply this cleaning procedure in the further steps of the protocol (i.e., sections 3 and 4).
- Using solvent-rinsed tweezers, sort the pellets by color in the following categories: white/transparent, whitish/yellowish, yellow/orange, amber/brown, and pigmented (e.g., red, green, blue, etc.)
- Gather 10 pellets of similar color randomly (i.e., plastic type not considered), which will constitute one replicate.
- Weigh the sample on an analytical balance and record the mass. At this stage, the samples can be put back in the fridge or freezer.
- To take into account the background contamination, perform a blank sample with each set of replicates (e.g., 1 blank for 5 replicates). To this end, apply the same protocol as described above, but do not add plastic pellets in the extraction cell. This blank sample will undergo the further steps of the protocol and be analyzed together with the samples.
- Switch on the PFE. Download the extraction method and warm-up the instrument to 60 °C. The details of the method are as follows:**
  - Set the temperature to 60 °C and the pressure to 100 bar.
  - Select one cycle with heat-up time of 1 min, a hold time of 25 min, and a discharge time of 2 min.
  - Set the solvent and gas (N<sub>2</sub>) flush times to 3 min each.
  - Select n-hexane as the extraction solvent.
- While the instrument is warming up, prepare the extraction cell as described below. If necessary, adapt the protocol to the supplier's instructions of your instrument:**
  - Place the bottom filter and the frit in the extraction cell. Close it and turn it over.
  - Fill approximately half the cell with cleaned quartz sand using a funnel.
  - Add the weighed sample (i.e., one replicate of 10 pellets). Frozen plastic pellets should be placed in the fridge overnight prior to extraction.
  - Add quartz sand up to 1 cm from the top of the cell. Take special care to use ultra clean quartz sand (or alternatively glass beads) since it is exposed to the same extraction conditions as the samples. To clean the sand, successively extract it in the PFE in analytical-grade dichloromethane and n-hexane, applying 2 or more cycles per solvent (e.g., 30 min at 100 °C under 100 bar). Alternatively, use an ultrasonic bath and/or rotating evaporator. Repeat the cleaning procedure, if necessary.
  - Insert the top filter in the cell and place the cell in the instrument.
- Place the collecting vessels in the instrument and start the extraction method (total run of about 35 min).

- When the method is completed, empty the extraction cell in a cleaned glass vessel (e.g., beaker, glass cell-culture dish) and retrieve the 10 pellets in the sand. Store them in a container until further analysis for plastic identification (e.g., zip bag or glass vial).

### 3. Concentration and Clean-up of the Extract

- Transfer the obtained extract (about 40 mL) from the collecting vessel to a glass tube and evaporate it to 1 mL in a rotating concentrator set to 35 °C for 20 min. Alternative methods could be used such as evaporation under nitrogen flow or rotating evaporator. The temperature and duration should be optimized accordingly.
- In the meantime, prepare the solid-phase extractor (SPE) by placing a waste tube in the rack and a cartridge filled with activated magnesium silicate sorbent (1 g) on the manifold in the close-valve position. The clean-up is based on the EPA method 3620C<sup>22</sup> as follows:**
  - Turn the vacuum on at the source and add 4 mL of hexane in the cartridge to activate the sorbent.
  - Open the valve and let the solvent pass through the entire sorbent bed. Then, close the valve and allow the sorbent to soak in hexane for 5 min.
  - Open the valve and let the solvent pass through, but close the valve before the sorbent dries off.
  - When the sample is concentrated, transfer it to the cartridge with a glass Pasteur pipette. Gently open the valve and let it pass through slowly. 1-2 drops per second is an appropriate speed.
  - Rinse the glass tube containing the extract with 0.5 mL of hexane and add it to the cartridge when the extract has passed through.
  - When the entire solvent has passed through, close the valve and turn off the vacuum.
  - Replace the waste tube with a collecting tube and use a clean solvent guide needle.
  - Add 9 mL of acetone/hexane (10/90, v/v) to the cartridge and turn on the vacuum at the source. Allow the sorbent to soak in the solvent for 1 min.
  - Open the valve and collect the entire eluate in the collecting tube.
- Place the collecting tube in the concentrator and evaporate the solvent for 9 min at 35 °C in order to reach 1 mL of eluate.
- Transfer the concentrated eluate into an amber autosampler vial with a glass Pasteur pipette. At this stage, the samples can be stored in the freezer prior to analysis.

### 4. Analysis of the Cleaned and Concentrated Extract

- Download the analytical method on the control software of the GC-μECD instrument (gas chromatograph equipped with a micro electron capture detector). The details of the method are as follows:**
  - Set the injector to splitless mode, its temperature to 250 °C, and the purge time to 1 min.
  - Set the flow of the carrier gas (He) to 1.5 mL min<sup>-1</sup>.
  - Program the column oven with the following temperature gradient: 60 °C hold for 1 min, ramp of 30 °C min<sup>-1</sup> to 200 °C, ramp of 5 °C min<sup>-1</sup> to 230 °C, ramp of 3 °C min<sup>-1</sup> to reach 250 °C, hold this temperature for 5 min.
  - Set the detector temperature to 300 °C and the back-up gas flow (N<sub>2</sub>) to 60 mL min<sup>-1</sup>.
- Place the vial containing the sample (cleaned and concentrated) in the autosampler rack and run the method (run time of 23.3 min). Inject 2 μL of sample.
- After the analysis, identify the different compounds on the chromatogram by their retention times and record the corresponding peak areas.
- Taking into account the recoveries (R) and the peak areas (A<sub>1</sub>), calculate the concentration (C<sub>1</sub>) of each OCP in the extract using the equations of the calibration curves as follows:

$$C_1 = \frac{\left[ \left( \frac{A_1 \times 100}{R} \right) - b \right]}{a}$$

where *b* is the intercept at the origin and *a* is the slope of the calibration equation,

$$C = ax + b$$

- Taking into account the mass (*m*) of the replicate (i.e., 10 pellets; see section 2.4) and the volume (*V*) of the final extract (i.e., 1 mL), calculate the concentration (C<sub>2</sub>) of each OCP adsorbed on the plastic pellets (i.e., ng of OCP per g of plastic pellet):

$$C_2 = \frac{V \times 10^{-3} \times C_1}{m}$$

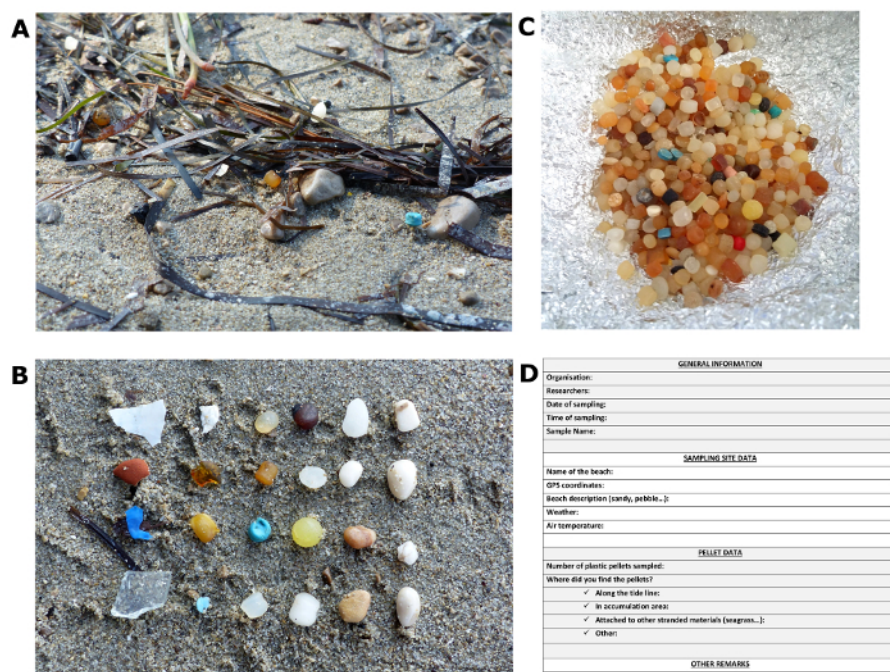
### 5. Plastic Type Identification

- Transfer the pellets in a glass Petri dish and place it in a plastic bag.
- Hold one pellet with tweezers and cut a slice of the pellet with a scalpel. The plastic bag prevents the loss of pellets during the cutting process.
- Clean the attenuated total reflectance (ATR) crystal of the FT-IR instrument with ethanol.
- Record a background spectrum.
- Place the fragment on the ATR crystal and screw the sample holder. The inner side of the piece must be in contact with the crystal.
- Scan the sample and record the spectrum.
- Identify the polymer constituting the plastic pellet by comparing the obtained spectrum to a spectra library. Although more time-consuming, the interpretation of the obtained spectra could be carried out manually as well, but most probably without reaching the degree of specificity achieved with a library search.

## Representative Results

Plastic pellets are usually found along the high and low tide lines of sandy beaches (**Figure 1A**). They can also stick to seagrass freshly stranded on beaches, after a storm for instance. They can occasionally be found on pebble and stony beaches in accumulation areas of stranded material.

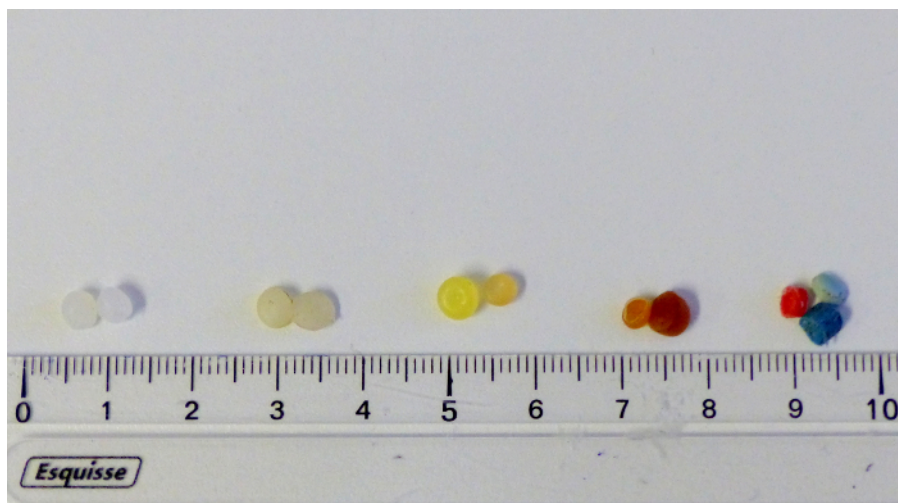
Plastic pellets are usually easily recognizable by their shape, size and color as shown in **Figure 1B** (see the two middle columns). They could be mistaken for tiny gravels (see columns 5 and 6), small biological fragments, or particles of different origins (see columns 1 and 2). Once in the laboratory, the suspicious items can be discarded. In case of doubt, it is possible to check the floatability of the samples in double distilled water. Gravels will sink whereas plastic pellets will mostly float. A sample of plastic pellets collected on a beach is shown in **Figure 1C** for illustration. An example of the identity beach form to be filled in the field is provided **Figure 1D**.



**Figure 1:** (A) Plastic resin pellets stranded on a sandy beach at the tide line. (B) Plastic resin pellets vs. gravel and other stranded materials. Fragments of different origins are presented in the 1<sup>st</sup> and 2<sup>nd</sup> columns from the left. Small gravel particles are aligned in columns 5 and 6. Plastic pellets are in the middle columns. (C) Sample of plastic resin pellets. (D) Example of an identity beach form. [Please click here to view a larger version of this figure.](#)

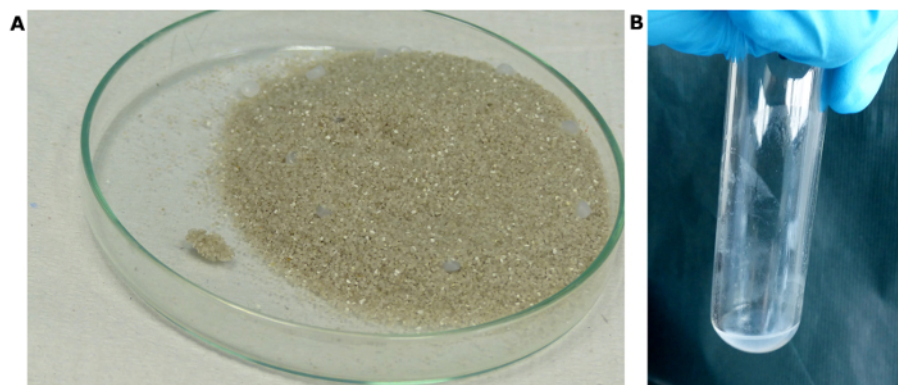
Whitish and yellowish pellets are usually predominant over other pellets, especially pigmented ones. However, some sampling sites present a particular profile and therefore it is advised to classify the plastic pieces by color (visual assessment) prior to extraction. A visual reference can be created to help sorting the pellets as presented on **Figure 2** (from left to right: white/transparent, whitish/yellowish, yellow/orange, amber/brown, and pigmented).





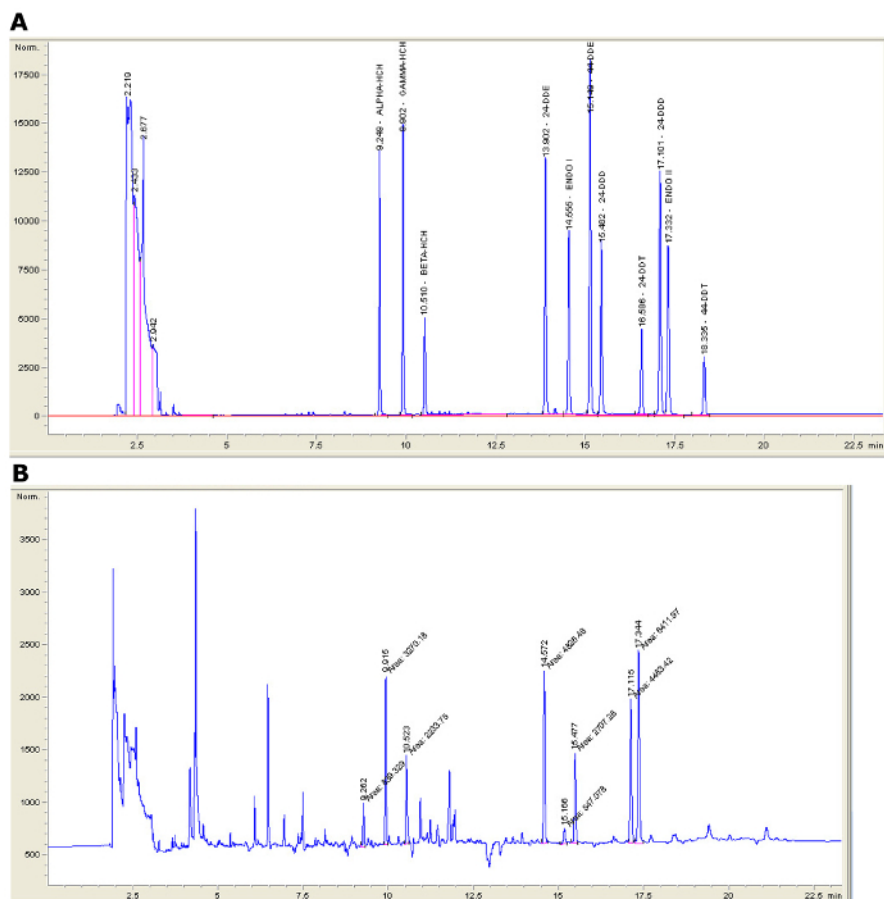
**Figure 2: Classification of plastic pellets by color, from left to right: white/transparent, whitish/yellowish, yellow/orange, amber/brown and pigmented.** [Please click here to view a larger version of this figure.](#)

It can happen that some pellets start to melt during the extraction process. Thus, quartz sand particles will stick on their surface after extraction. For instance, in **Figure 3A**, the isolated pellet on the left of the Petri dish has sand particles sticking on its surface due to melting. This often occurs with ethylene/vinyl acetate copolymers due to their low melting point compared to other plastic polymers such as PE and PP. Exceptionally, the melting process can be too severe and the extract will appear milky (**Figure 3B**). In this case, it is advised to discard the sample immediately after extraction. This extract would clog the sorbent of the SPE cartridge.



**Figure 3: (A)** The isolated pellet on the left of the Petri dish has sand particles sticking on its surface due to melting. **(B)** Extraction with polymer break down. The melting process can render the extract to appear milky. [Please click here to view a larger version of this figure.](#)

As a first approach, spiked virgin pellets were prepared in order to optimize the extraction step and assess its repeatability. As can be seen from **Figure 4A**, all 11 OCPs were extracted applying the described protocol. In addition, **Figure 4B** illustrates the analysis result of OCPs extracted from pellets sampled on a beach at the Adriatic coast. In this case, 8 OCPs out of 11 were detected. The chromatographic peak identification is based on the retention times obtained from the injection of OCPs individual standard solutions. A deviation from standard peak retention time of 0.1% is accepted as the maximum. The calculation of OCPs concentrations is based on the analysis of standard solutions. Calibration equations and recoveries from the SPE and concentration steps must be determined for each studied compound prior to sample analysis (**Table 1**).

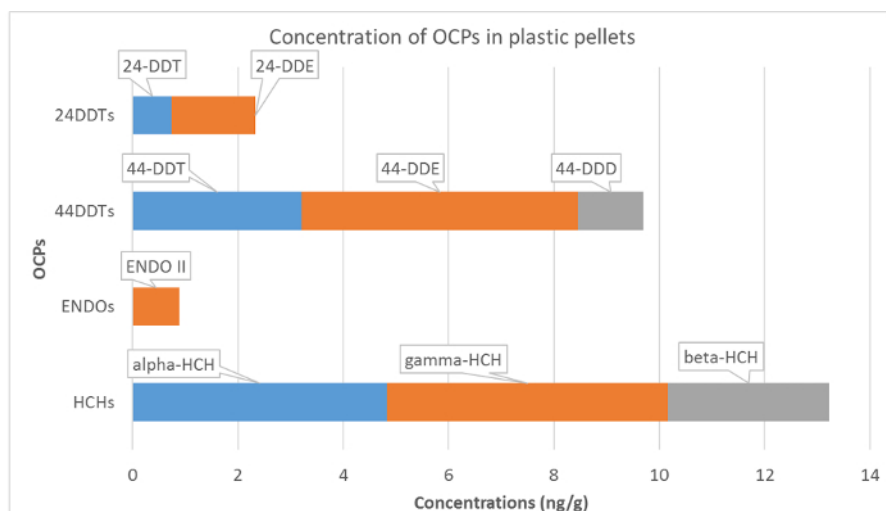


**Figure 4:** (A) Chromatogram of OCPs extracted from spiked virgin PE pellets. (B) Chromatogram of OCPs extracted from pellets sampled on the Adriatic coast. [Please click here to view a larger version of this figure.](#)

Compounds	Tr (min)	Calibration curve equation	R2	Recovery (%)
a-HCH	9.25	$y = 1836x - 315$	0.9992	99
g-HCH	9.92	$Y = 2055x - 158$	0.9996	96
b-HCH	10.45	$Y = 772x + 58$	0.9993	78
24-DDE	13.90	$y = 2611x + 262$	0.9999	76
Endosulphan I	14.50	$y = 2015x + 280$	0.9999	74
44-DDE	15.16	$y = 3942x - 427$	0.9988	82
24-DDD	15.52	$y = 1822x + 157$	0.9999	94
24-DDT	16.64	$y = 962x - 93$	0.9965	75
44-DDD	17.11	$y = 2617x + 44$	0.9992	86
Endosulphan II	17.30	$y = 2212x + 123$	0.9995	102
44-DDT	18.32	$y = 725x - 80$	0.9955	96

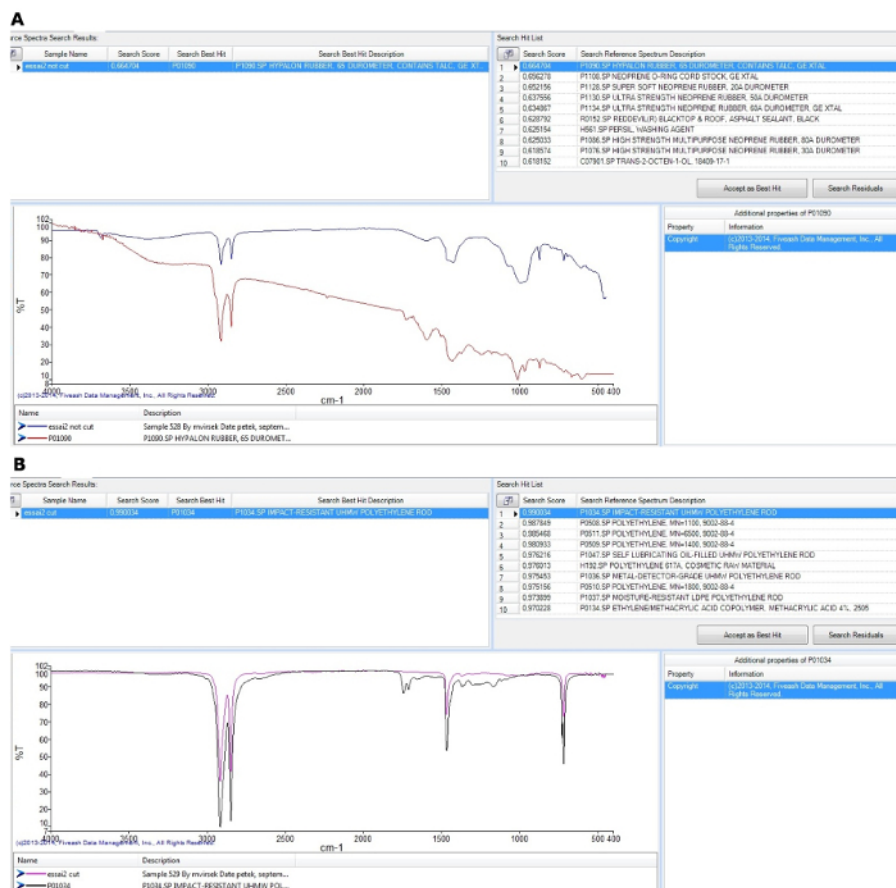
**Table 1:** Example of calibration and recovery results obtained for the 11 studied OCPs.

A representative concentration is determined for each sampling location by analyzing at least 3 replicates of 10 pellets and taking the median value. The latter is preferred to the average value due to the dispersion of the results<sup>4</sup>. **Figure 5** shows an example of results based on 5 replicates.



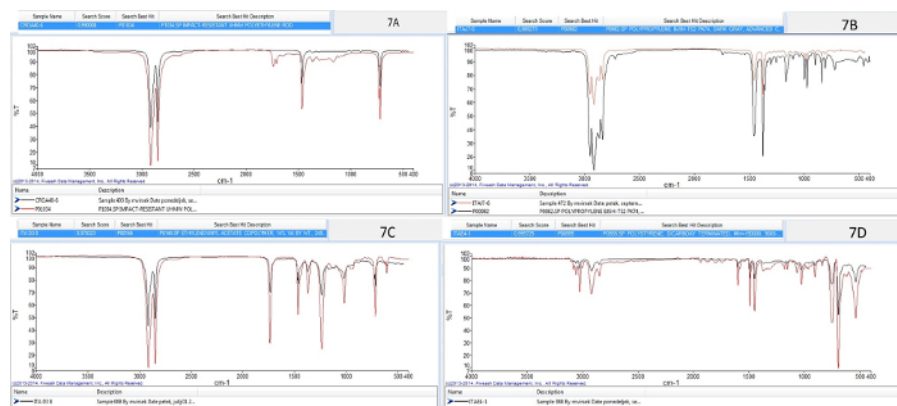
**Figure 5: Median concentration of OCPs extracted from pellets sampled on the Adriatic coast.** The data shows an example of results based on 5 replicates. [Please click here to view a larger version of this figure.](#)

The chemical analysis of the plastic is carried out on an ATR-FT-IR spectrometer. The measurement is performed on the inner side of a pellet slice. Plastic pieces are covered by biofilms and/or by adherent layer(s), which can interfere in the IR spectra of the sample. Thus, cutting the pellets allows an easier identification of the polymer than processing uncut items, because the ATR crystal is in contact with less contaminated material. The chemical composition analysis results of an uncut pellet are shown in **Figure 6A**. The item was identified as rubber with a probability of about 66% at the highest. **Figure 6B** presents the results obtained from a slice of the same pellet, which was ultimately shown to be made of PE with a probability of 99%. The second measurement was performed on the inner side of the fragment.



**Figure 6: (A)** FT-IR spectrum of the uncut pellet and best hit results from the spectra library. **(B)** FT-IR spectrum of the pellet slice and best hit results from the spectra library. [Please click here to view a larger version of this figure.](#)

PE, as identified in **Figure 7A**, is the most common polymer type found in plastic pellets, followed by PP (**Figure 7B**). Ethylene/vinyl acetate copolymer is the 3<sup>rd</sup> most common plastic type usually identified (**Figure 7C**). Pellets made of PS can occasionally also be found (**Figure 7D**). An example of plastic type identification for a replicate of 10 pellets is given in **Table 2**. As can be seen, the sample consists of 70% by PE.



**Figure 7:** FT-IR spectrum and best hit results of a pellet identified as (A) PE (99.0%); (B) PP (98.9%); (C) ethylene/vinyl acetate copolymer (97.0%); and (D) PS (99.6%). [Please click here to view a larger version of this figure.](#)

Sample Name	Search Score	Search Best Hit	Search Best Hit Description
Sample 1-1	0.990764	P01034	P1034.SP IMPACT-RESISTANT UHMW POLYETHYLENE ROD
Sample 1-2	0.992768	P00508	P0508.SP POLYETHYLENE, MN=1100, 9002-88-4
Sample 1-3	0.990528	P01037	P1037.SP MOISTURE-RESISTANT LDPE POLYETHYLENE ROD
Sample 1-4	0.956303	P00561	P0561.SP POLYSTYRENE, MONOCARBOXY TERMINATED, MW=200000, 9003-53-6
Sample 1-5	0.988493	P00147	P0147.SP ETHYLENE/VINYL ACETATE COPOLYMER, 18% VA BY WT., 24937-78-8
Sample 1-6	0.990185	P01046	P1046.SP RIGID HDPE POLYETHYLENE ROD
Sample 1-7	0.988167	P01034	P1034.SP IMPACT-RESISTANT UHMW POLYETHYLENE ROD
Sample 1-8	0.969821	P00546	P0546.SP POLYPROPYLENE, ISOTACTIC, TG=-26, 9003-07-0
Sample 1-9	0.991779	P01036	P1036.SP METAL-DETECTOR-GRADE UHMW POLYETHYLENE ROD
Sample 1-10	0.988388	P01046	P1046.SP RIGID HDPE POLYETHYLENE ROD

**Table 2:** Polymer identification results of a pool of 10 pellets.

## Discussion

Most studies focusing on organic contaminants associated to plastic pellets have relied on classical extraction methods of the adsorbed chemicals. The Soxhlet apparatus is the most widely used technique with typical extraction times ranging from 12 to 24 h and with high consumption of organic solvents (*i.e.*, from 100 to 250 mL per extraction)<sup>23</sup>. Maceration extractions require a long contact time between the sample and the organic solvent (*e.g.*, 6 days)<sup>4</sup> and can be hastened by adding an ultrasonication step. In contrast, pressurized fluid extraction, as described in this study, is an efficient way to rapidly extract analytes from solid or semi-solid matrices under high pressure and temperature using a reduced amount of solvent (*e.g.*, 40 mL). Although it is commonly used as an alternative to the Soxhlet method, this technique has rarely been employed in the field of microplastics<sup>14</sup>. One of the limitations linked to the application of this technique to the analysis of plastic fragments is the potential melting of polymers, which are then difficult to remove from the extract and often make its analysis impossible. This issue is not encountered when extracting organics from homogeneous matrices. In this case, the extraction temperature is set according to the polymer type of the plastic sample. Microplastic samples are composed of a heterogeneous mixture of items made of various polymer types in different degradation states, which often cause the early melting of the plastic. Thus, the temperature in the PFE cell must be optimized to allow the



extraction of OCPs regardless of the polymer type and its degradation state. In this work, a temperature of 60 °C together with a long hold time were found to be a good compromise between extraction efficiency and melting issues. Only rubber and aged ethylene/vinyl acetate copolymer are prone to melting, but these polymers are usually present at such low amounts in the sample that they do not affect extraction.

In many studies<sup>4,8,13,16,18</sup>, only aged PE pellets are analyzed for their adsorbed organic contaminant content. Because of their surface properties, this category of polymers has a greater affinity to adsorb environmental pollutants than other type of pellets and they are the predominant polymer class<sup>4</sup>. However, some sampling locations present a special profile with an abundance of less aged pellets (*i.e.*, white or transparent) and/or a higher variety in polymer types than commonly found. Thus, a different approach is suggested here to avoid a possible overestimation of the organic contaminant levels. The classification of plastic pellets is based on color rather than on polymer type. Moreover, the identification of the plastic type can still be carried out after the extraction step. By proceeding in this order, the risk of sample contamination during the polymer chemical analysis is lowered and the plastic identification process can be facilitated by cutting the pellets, as previously explained. Extracting organic contaminants from items that are mistaken for plastic pellets would be the main limitation of this methodology. However, it can be underlined that only a negligible fraction (*i.e.*, less than 0.5%) of the sampled pellets is shown not to be made of plastic polymer after chemical analysis.

This protocol was developed for the determination of OCPs adsorbed on plastic pellets. However, it can be adapted for detection of other categories of organic contaminants usually found associated to microplastics such as polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs), as well as plastic softeners or additives. To this end, the clean-up step would have to be further optimized by eluting the sorbent with several successive solvents of different polarities<sup>4,10</sup>. To some extent, the extraction solvent composition could also be modified, by adding a fraction of dichloromethane and/or acetone to hexane, for instance. Finally, new analytical methods must be developed specially for the compounds to be investigated. Although Gas Chromatography-Electron Capture Detector (GC-ECD) is a sensitive technique, its selectivity for halogenated compounds limits its application to other classes of compounds. Moreover, peak identification is only based on retention times, which can lead to misinterpretation of chromatograms. To lower the risk of misidentification, a deviation from standard solution retention times of only 0.1% is accepted. Gas chromatography equipped with a mass spectrometer (GC-MS) is an appropriate technique for validating the peak identification. It could be run in parallel to GC-ECD or used as a single analysis method if its sensitivity allows the quantification of trace concentrations.

This methodology focuses on resin pellets, but it could be further optimized for the analysis of other microplastic categories. However, the sorting of plastic fragments from environmental samples (*e.g.*, sea surface, sediment, or biota) is more challenging than the one of pellets and a visual identification is not appropriate. Thus, the chemical analysis of the polymers should be performed prior to extraction. Knowing that microplastic sizes range from 5 mm to a few hundred µm (*e.g.*, 300 µm), the analysis should be performed on a micro ATR-FTIR spectrometer, which is adapted for the measurements of small particles<sup>19,20</sup>. Moreover, the separation of microplastics from environmental samples usually requires the use of solvents (*e.g.*, ethanol) and/or strong acids or bases (*e.g.*, acid digestion of tissues), which can desorb and/or degrade the organic contaminants associated to the particles. Thus, alternative separation techniques should be developed, which would preserve the chemicals. In addition, it should be underlined that the quantity of microplastics detected in sea surface and biota is often insufficient for performing quantitative analyses of organic compounds. This protocol is adapted to process plastic fragments visible to the naked eye and made of hard polymers. It is not likely to work on soft materials or extremely small items (*i.e.*, <1 mm). Thus, the microplastic categories of films, filaments, and foams should be discarded from the samples. Nevertheless, small microplastic pieces could be analyzed for their organic contaminant content and their polymer type. In this case, it is advised to cut the items in small particles of a few mm prior to extraction or FT-IR analysis.

## Disclosures

The authors have nothing to disclose.

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